

# TAHC - SEPTEMBER 2005 REPORT

## BOVINE AND SMALL RUMINANT SEMEN

### Article 3.2.1.1.

#### **General considerations**

The purposes of official sanitary control of semen production are to:

1. maintain the health of animals on an *artificial insemination centre* at a level which permits the international distribution of semen with a negligible risk of infecting other animals or humans with pathogens transmissible by semen;
2. ensure that semen is hygienically collected, processed and stored.

Standards for diagnostic tests are described in the *Terrestrial Manual*.

### Article 3.2.1.2.

#### **Conditions applicable to artificial insemination centres**

1. The *artificial insemination centre* is comprised of:
  - a) animal accommodation areas (including one isolation facility for sick animals) and a semen collection room, these two premises hereon designated as semen collection facilities; accommodation areas should be species specific where relevant;
  - b) a semen laboratory and semen storage areas;
  - c) administration offices.

A *quarantine station* may also be attached to the centre, provided that it is on a different location from that of those two first parts.

2. The centre should be officially approved by the *Veterinary Administration*.
3. The centre should be under the supervision and control of the *Veterinary Authority* which will be responsible for regular audits, at an interval of no more than 6 months, of protocols, procedures and prescribed records on the health and welfare of the animals in the centre and on the hygienic production, storage and dispatch of semen.
4. The centre should be under the direct supervision and control of a veterinarian designated by the *artificial insemination centre* and accredited by the *Veterinary Administration* for relevant official tasks.

### Article 3.2.1.3.

#### **Conditions applicable to semen collection facilities**

1. The semen collection facilities should include separate and distinct areas for accommodating resident animals, for semen collection, for feed storage, for manure storage, and for the isolation of ~~suspect~~ animals suspected of being infected.

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2. Only animals associated with semen production should be permitted to enter the semen collection facilities. Other species of animals may be resident at the centre, if necessary for the movement or handling of the donors and teasers or for security, but contact with the donors and teasers should be minimised. All animals resident at the semen collection facilities must meet the minimum health requirements for donors.
3. The donors and teasers should be adequately isolated to prevent the transmission of diseases from farm livestock and other animals. Measures should be in place to prevent the entry of wild animals susceptible to OIE-listed ruminant diseases transmissible via semen.
4. Personnel at the centre should be technically competent and observe high standards of personal hygiene to preclude the introduction of pathogenic organisms. Special protective clothing and footwear for use only at the semen collection facilities should be provided and worn at all times inside.
5. Visitors to the semen collection facilities should be kept to a minimum, and visits should be subject to formal authorisation and control. Equipment for use with the livestock should be dedicated to the semen collection facilities or disinfected prior to entry. All equipment and tools brought on to the premises must be examined and treated if necessary to ensure that they cannot introduce disease.
6. *Vehicles* used for transport of animals to and from the semen collection facilities should not be allowed to enter the facilities.
7. The semen collection area should be cleaned daily after collection. The animals' accommodation and semen collection areas should be cleaned and disinfected at least once a year.
8. Fodder introduction and manure removal should be done in a manner which poses no significant animal health risk.

### Article 3.2.1.4.

#### **Conditions applicable to semen laboratories**

1. The semen laboratory should be physically separated from the semen collection facilities, and include separate areas for artificial vagina cleaning and preparation, semen evaluation and processing, semen pre-storage and storage. Entry to the laboratory should be prohibited to unauthorised personnel.
2. The laboratory personnel should be technically competent and observe high standards of personal hygiene to preclude the introduction of pathogenic organisms during semen evaluation, processing and storage.
3. Visitors to the laboratory should be kept to a minimum, and visits should be subject to formal authorisation and control.
4. The laboratory should be constructed with materials that permit effective cleaning and *disinfection*.
5. The laboratory should be regularly cleaned. Work surfaces for semen evaluation and processing should be cleaned and disinfected at the end of each workday.

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6. The laboratory should be treated against rodents and insects on a regular basis as needed to control these pests.
7. The storage rooms and individual semen containers should be easy to clean and disinfect.
8. Only semen collected from donors having a health status equivalent to or better than the donors at the semen collection facilities should be processed in the laboratory.

Article 3.2.1.5.

### Conditions applicable to testing of bulls and teaser animals

Bulls and teaser animals ~~can~~ should enter an *artificial insemination centre* only if they fulfil the following requirements ~~laid down by the Veterinary Administration.~~

#### 1. Pre-quarantine

The animals should comply with the following requirements prior to entry into isolation at the *quarantine station*.

##### a) Bovine brucellosis

The animals should comply with point 3 or 4 of Article 2.3.1.5. of the *Terrestrial Code*.

##### b) Bovine tuberculosis

The animals should comply with point 2, 3 or 4 of Article 2.3.3.4. of the *Terrestrial Code*.

##### c) Bovine viral diarrhoea-mucosal disease (BVD-MD)

The animals should be subjected to the following tests:

- i) a virus isolation test or a test for virus antigen, with negative results;
  - ii) a serological test to determine the serological status of every animal.
- ##### d) Infectious bovine rhinotracheitis-infectious pustular vulvovaginitis (IBR/IPV)

If the *artificial insemination centre* is to be considered as IBR/IPV free, the animals should either:

- i) come from an IBR/IPV free herd as defined in Article 2.3.5.3.; or
- ii) be subjected, with negative results, to a serological test for IBR/IPV on a blood sample.

##### e) Bluetongue

The animals should comply with Article 2.2.13.6., 2.2.13.7. or 2.2.13.8., depending on the bluetongue status of the country of origin of the animals.

#### 2. Testing in the quarantine station prior to entering the semen collection facilities

Prior to entering the semen collection facilities of the *artificial insemination centre*, bulls and teaser animals should be kept in a *quarantine station* for at least 28 days. The animals should be subjected to diagnostic tests as described below a minimum of 21 days after entering the

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*quarantine station*, except for *Campylobacter fetus* subsp. *venerealis* and *Trichomonas foetus*, for which testing may commence after 7 days in quarantine. All the results should be negative except in the case of BVD-MD antibody serological testing (see point 2b)i) below).

a) Bovine brucellosis

If the country is not free from brucellosis, the animals should be subjected to a serological test with negative results.

b) BVD-MD

i) All animals should be tested for viraemia as described in point 1c) above.

Only when all the animals in quarantine test negative for viraemia may the animals enter the semen collection facilities upon completion of the 28-day quarantine period.

ii) After 21 days in quarantine, all animals should be subjected to a serological test to determine the presence or absence of BVD-MD antibodies.

iii) Only if no sero-conversion occurs in the animals which tested seronegative before entry into the *quarantine station*, may any animal (seronegative or seropositive) be allowed entry into the semen collection facilities.

iv) If sero-conversion occurs, all the animals that remain seronegative should be kept in quarantine over a prolonged time until there is no more seroconversion in the group for a period of 3 weeks. Serologically positive animals may be allowed entry into the semen collection facilities.

c) *Campylobacter fetus* subsp. *venerealis*

i) Animals less than 6 months old or kept since that age only in a single sex group prior to quarantine should be tested once on a preputial specimen, with a negative result.

ii) Animals aged 6 months or older that could have had contact with females prior to quarantine should be tested three times at weekly intervals on a preputial specimen, with a negative result in each case.

d) *Trichomonas foetus*

i) Animals less than 6 months old or kept since that age only in a single sex group prior to quarantine, should be tested once on a preputial specimen, with a negative result.

ii) Animals aged 6 months or older that could have had contact with females prior to quarantine should be tested three times at weekly intervals on a preputial specimen, with a negative result in each case.

e) IBR/IPV

If the *artificial insemination centre* is to be considered as IBR/IPV free, the animals should be subjected, with negative results, to a diagnostic test for IBR/IPV on a blood sample. If any animal tests positive, the animal should be removed immediately from the *quarantine station* and the other animals of the same group should remain in quarantine and be retested, with negative results, not less than 21 days after removal of the positive animal.

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f) Bluetongue

The animals should comply with Article 2.2.13.9., 2.2.13.10. or 2.2.13.11., depending on the bluetongue status of the country of origin of the animals.

3. Testing for BVD-MD prior to the initial dispatch of semen from each serologically positive bull

Prior to the initial dispatch of semen from BVD-MD serologically positive bulls, a semen sample from each animal should be subjected to a virus isolation or virus antigen ELISA test for BVD-MD. In the event of a positive result, the bull should be removed from the centre and all of its semen destroyed.

4. Testing of frozen semen for IBR/IPV in artificial insemination centres not considered as IBR/IPV free

Each aliquot of frozen semen should be tested as per Article 2.3.5.7.

5. Testing programme for bulls and teasers resident in the semen collection facilities

All bulls and teasers resident in the semen collection facilities should be tested at least annually for the following diseases, with negative results, where the country of origin is not free:

- a) Bovine brucellosis
- b) Bovine tuberculosis
- c) BVD-MD

Animals negative to previous serological tests should be retested to confirm absence of antibodies.

Should an animal become serologically positive, every ejaculate of that animal collected since the last negative test should be either discarded or tested for virus with negative results.

d) *Campylobacter fetus* subsp. *venerealis*

- i) A preputial specimen should be cultured.
- ii) Only bulls on semen production or having contact with bulls on semen production need to be tested. Bulls returning to collection after a lay off of more than 6 months should be tested not more than 30 days prior to resuming production.

e) Bluetongue

The animals should comply with the provisions referred to in Article 2.2.13.9., 2.2.13.10. or 2.2.13.11., depending on the bluetongue status of the country of origin of the animals.

f) *Trichomonas foetus*

- i) A preputial specimen should be cultured.

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- ii) Only bulls on semen production or having contact with bulls on semen production need to be tested. Bulls returning to collection after a lay off of more than 6 months should be tested not more than 30 days prior to resuming production.
  - g) IBR/IPV
- If the *artificial insemination centre* is to be considered as IBR/IPV free, the animals should comply with the provisions in point 2)c) of Article 2.3.5.3.

Article 3.2.1.6.

### Conditions applicable to testing of rams/bucks and teaser animals

Rams/bucks and teaser animals can enter an *artificial insemination centre* only if they fulfil the following requirements ~~laid down by the Veterinary Administration.~~

#### 1. Pre-quarantine

The animals should comply with the following requirements prior to entry into isolation at the *quarantine station*.

- a) Caprine and ovine brucellosis

The animals should comply with Article 2.4.2.6.

- b) Ovine epididymitis

The animals should comply with Article 2.4.1.3.

- c) Contagious agalactia

The animals should comply with points 1 and 2 of Article 2.4.3.1.

- d) Peste des petits ruminants

The animals should comply with points 1, 2, and 4 ~~and~~ 5 of Article 2.4.9.7.

- e) Contagious caprine pleuropneumonia

The animals should comply with Article 2.4.6.5. or Article 2.4.6.7., depending on the CCPP status of the country of origin of the animals.

- ~~f) Caseous lymphadenitis~~

~~The animals should be free from clinical signs for the past 12 months.~~

- g) Paratuberculosis

The animals should be free from clinical signs for the past 2 years.

- h) Scrapie

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If the animals do not originate from a scrapie free country or ~~zone~~ as defined in Article 2.4.8.3., the animals should comply with points 1 and 2 of Article 2.4.8.8.

i) Maedi-visna

The animals should comply with Article 2.4.5.2.

j) Caprine arthritis/encephalitis

The animals should comply with Article 2.4.4.2.

k) Bluetongue

The animals should comply with Article 2.2.13.6., 2.2.13.7. or 2.2.13.8., depending on the bluetongue status of the country of origin of the animals.

l) Tuberculosis

In the case of goats, the animals should be subject to a single or comparative tuberculin test, with negative results.

~~m) Border disease~~

~~The animals should be subject to a viral agent isolation test with negative results.~~

### 2. Testing in the quarantine station prior to entering the semen collection facilities

Prior to entering the semen collection facilities of the *artificial insemination centre*, rams/bucks and teasers should be kept in a *quarantine station* for at least 28 days. The animals should be subjected to diagnostic tests as described below a minimum of 21 days after entering the *quarantine station*, with negative results:

a) Caprine and ovine brucellosis

The animals should be subject to testing as described in point 1 b) or c) of Article 2.4.2.8.

b) Ovine epididymitis

The animals and semen should be subject to testing as described in points 1 d) and 2 of Article 2.4.1.4.

c) Maedi-visna and caprine arthritis/encephalitis ~~or CAE~~

The animals should be subjected to a serological test.

d) Bluetongue

The animals should comply with the provisions referred to in Article 2.2.13.9., 2.2.13.10. or 2.2.13.11., depending on the bluetongue status of the country of origin of the animals.

3. Testing programme for rams/bucks and teasers resident in the semen collection facilities

All rams/bucks and teasers resident in the semen collection facilities should be tested at least annually for the following diseases, with negative results, where the country of origin is not free:

- a) caprine and ovine brucellosis;
- b) ovine epididymitis;
- c) Maedi-visna and caprine arthritis/encephalitis ~~or CAE~~;
- d) tuberculosis (for goats only);
- e) bluetongue.

Article 3.2.1.7.

**General considerations for hygienic collection and handling of semen**

Observation of the recommendations described in the Articles below will very significantly reduce the likelihood of the semen being contaminated with common bacteria which are potentially pathogenic.

Article 3.2.1.8.

**Conditions applicable to the management of bulls, rams and bucks**

The objective is to keep the animals in a satisfactory state of cleanliness, particularly of the lower thorax and abdomen.

- 1. Whether on pasture or housed, the animal should be kept under hygienic conditions. If housed, the litter must be kept clean and renewed as often as necessary.
- 2. The coat of the animal should be kept clean.
- 3. For bulls, the length of the tuft of hairs at the preputial orifice, which is invariably soiled, should be cut to about 2 cm. The hair should not be removed altogether, because of its protective role. If cut too short, irritation of the preputial mucosa may result because these hairs aid the drainage of urine.
- 4. The animal should be brushed regularly, and where necessary on the day before semen collection, paying special attention to the underside of the abdomen.
- 5. In the event of obvious soiling, there should be careful cleaning, with soap or a detergent, of the preputial orifice and the adjoining areas, followed by thorough rinsing and drying.
- 6. When the animal is brought into the collection area, the technician must make sure that it is clean, and that it is not carrying any excessive litter or particles of feed on its body or its hooves, for such materials are always heavily contaminated.

Measures similar to the above should be adapted to rams and bucks.



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### Article 3.2.1.9.

#### Conditions applicable to the collection of semen

1. The floor of the mounting area should be easy to clean and to disinfect. A dusty floor should be avoided.
2. The hindquarters of the teaser, whether a dummy or a live teaser animal, must be kept clean. A dummy must be cleaned completely after each period of collection. A teaser animal must have its hindquarters cleaned carefully before each collecting session. The dummy or hindquarters of the teaser animal should be sanitized after the collection of each ejaculate. Disposable plastic covers may be used.
3. The hand of the person collecting the semen must not come into contact with the animal's penis. Disposable gloves should be worn by the collector and changed for each collection.
4. The artificial vagina must be cleaned completely after each collection. It should be dismantled, its various parts washed, rinsed and dried, and kept protected from dust. The inside of the body of the device and the cone should be disinfected before re-assembly using approved *disinfection* techniques such as those involving the use of 70° ethyl or 98-99° isopropyl alcohol, ethylene oxide or steam. Once re-assembled, it should be kept in a cupboard which is regularly cleaned and disinfected.
5. The lubricant used should be clean. The rod used to spread the lubricant must be clean and should not be exposed to dust between successive collections.
6. The artificial vagina should not be shaken after ejaculation, otherwise lubricant and debris may pass down the cone to join the contents of the collecting tube.
7. When successive ejaculates are being collected, a new artificial vagina should be used for each mounting. The vagina should also be changed when the animal has inserted its penis without ejaculating.
8. The collecting tubes should be sterile, and either disposable or sterilised by autoclaving or heating in an oven at 180°C for at least 30 minutes. They should be kept sealed to prevent exposure to the environment while awaiting use.
9. After semen collection, the tube should be left attached to the cone and within its sleeve until it has been removed from the collection room for transfer to the laboratory.

### Article 3.2.1.10.

#### Conditions applicable to the handling of semen and preparation of semen samples in the laboratory

1. Diluents
  - a) All receptacles used should have been sterilised.
  - b) Buffer solutions employed in diluents prepared on the premises should be sterilized by filtration (0.22 µm) or by autoclaving (121°C for 30 minutes) or be prepared using sterile water before adding egg yolk (if applicable) or equivalent additive and antibiotics.

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- c) If the constituents of a diluent are supplied in commercially available powder form, the water used must have been distilled or demineralised, sterilized (121°C for 30 minutes or equivalent), stored correctly and allowed to cool before use.
- d) When egg yolk is used, it should be separated from eggs using aseptic techniques. Alternatively, commercial egg yolk prepared for human consumption or egg yolk treated by, for example, pasteurisation or irradiation to reduce bacterial contamination, may be used. Other additives must also be sterilized before use.
- e) Diluent should not be stored for more than 72 hours at +5°C before use. A longer storage period is permissible for storage at -20°C. Storage vessels should be stoppered.
- f) A mixture of antibiotics should be included with a bactericidal activity at least equivalent to that of the following mixtures in each ml of frozen semen: either gentamicin (250 µg), tylosin (50 µg), lincomycin-spectinomycin (150/300 µg) or penicillin (500 IU), streptomycin (500 µg), lincomycin-spectinomycin (150/300 µg).

The names of the antibiotics added and their concentration should be stated in the *international veterinary certificate*.

### 2. Procedure for dilution and packing

- a) The tube containing freshly collected semen should be sealed as soon as possible after collection, and kept sealed until processed.
- b) After dilution and during refrigeration, the semen should also be kept in a stoppered container.
- c) During the course of filling receptacles for dispatch (such as insemination straws), the receptacles and other disposable items should be used immediately after being unpacked. Materials for repeated use should be sterilised with alcohol, ethylene oxide, steam or other approved sterilisation techniques.
- d) If sealing powder is used, care should be taken to avoid its being contaminated.

### 3. Conditions applicable to the storage of semen

Semen for export should be stored separately from other genetic material not meeting these guidelines in fresh liquid nitrogen in sterilised/sanitised flasks before being exported.

Semen straws should be sealed and code marked in line with the international standards of the International Committee for Animal Recording (ICAR)\*.

Prior to export, semen straws or pellets should be identified and placed into new liquid nitrogen in a new or sterilised flask or container under the supervision of an Official Veterinarian. The contents of the container or flask should be verified by the Official Veterinarian prior to sealing. Containers should be sealed with an official numbered seal under the responsibility of the Veterinary Administration before export and accompanied by an international veterinary certificate listing the contents and the number of the official seal.

- \* The ICAR international standards on straws are contained in *Recording Guidelines - Appendices* to the international agreement of recording practices. Section 9, Appendix B relating to semen straw identification.